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Biogenesis of Organelles and Membrane Proteins

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Biogenesis of mitochondrial proteins

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Eukaryotic cells, in contrast to procaryotic cells, are divided into a number of compartments, each surrounded by one or more membranes. As a consequence of creating this compartmentalization (i.e. organelles), eukaryotic cells have evolved a host of new capabilities compared with simple prokaryotic systems. Each compartment contains a unique set of proteins that enables it to carry out specific functions. Thus the accurate and efficient delivery of each protein to its correct compartment is an important step in the maintenance and development of the cell. Here we discuss the biogenesis of mitochondria, in particular with reference to the events involved in the import of proteins into these organelles (for other reviews see Zimmermann, 1986; Nicholson & Neupert, 1988).

Less than 10% of the mitochondrial proteins are encoded for by the mitochondrial genome; the remainder being specified by nuclear genes (Neupert & Schatz, 1981). In general, these proteins are synthesized on free ribosomes as precursors, containing *N*-terminal extensions, which are released into the cytoplasm (Hallermayer *et al.*, 1977). These precursors are subsequently imported into one of the four mitochondrial subcompartments: namely, outer membrane, intermembrane space, inner membrane or matrix. Although binding and import of mitochondrial precursors proteins does not depend on a coupled translational event, it is still quite possible that under certain physiological conditions *in vivo*, mitochondrial precursors could be imported in a co-translational manner (Schleyer & Neupert, 1985).

The *N*-terminal prepiece present on most mitochondrial precursor proteins contains the necessary information to target the protein to mitochondria (Hurt *et al.*, 1984*a*, *b*; van Loon & Young, 1986). As will be discussed later, these prepieces can also play an important role in subsequent intramitochondrial sorting (van Loon *et al.*, 1987; Hartl *et al.*, 1986, 1987). It is important to note, however, that some precursor proteins do not contain *N*-terminal extensions. For example, the outer membrane proteins porin and the 70 kDa protein from yeast (Mihara & Sato, 1985; Kleene *et al.*, 1987; Riezman, 1983; Hase *et al.*, 1984) and cytochrome *c* of the intermembrane space (Zimmermann *et al.*, 1981; Stuart *et al.*, 1987) are not proteolytically processed upon import. Clearly specific targeting information for these precursor proteins must reside within the mature part of the polypeptide itself, although it is not known whether such targeting information operates on the level of the primary structure or if it is dependent on a critical conformational arrangement of the protein (Nicholson *et al.*, 1987).

Proteinaceous components (import receptors) on the outer surface of the outer mitochondrial membrane are involved in the initial recognition process of the precursors (Hennig & Neupert, 1981; Zwizinski *et al.*, 1984; Pfaller & Neupert, 1987). The high-affinity binding of precursors to both porin and cytochrome *c* have been studied in detail (Hennig *et al.*, 1983; Pfaller & Neupert, 1987). In the case of porin, Scatchard analysis has indicated the existence of high-affinity binding sites present at 5–10 pmol/mg of mitochondrial protein and having a K_a of $(1-5)\times 10^8$ M^{-1}, while binding studies with cytochrome *c* indicated that there were 90 pmol of high-affinity binding sites per mg of mitochondrial protein. These sites had an association constant of 2.2×10^7. The binding of porin to mitochondria was sensitive to pretreatment of mitochondria with very low concentrations of trypsin, indicating that the binding sites are of a proteinaceous nature. These high-affinity binding sites could be solubilized with detergent and reconstituted into liposomes. It has also been suggested that this porin 'receptor' may also be involved in mediating the import of other precursor proteins such as the ADP/ATP carrier, an inner membrane protein.

Transport of precursors across the two mitochondrial membranes occurs in a single step at contact sites between the outer and inner membranes (Schleyer & Neupert, 1985; Schwaiger *et al.*, 1987). These contact sites have been shown to be stable structures the formation of which was not mediated by the presence of the precursor proteins themselves. Precursor proteins trapped in these contact sites (translocation intermediates) have been labelled by immunocytochemical techniques and visualized by electron microscopy (Schwaiger *et al.*, 1987). The insertion into and translocation across the outer and inner membranes are dependent on an electrochemical potential ($\Delta\psi$) across the inner membrane (Schleyer *et al.*, 1982; Gasser *et al.*, 1982; Pfanner & Neupert, 1986). It is possible that the $\Delta\psi$ produces an electrophoretic driving force on the positively charged parts of the precursor protein, which serves to mediate penetration into or through the inner membrane (Pfanner & Neupert, 1986; Roise *et al.*, 1986).

The role of nucleoside triphosphates (NTPs) in the transport of proteins into the mitochondrion has also been investigated (Pfanner & Neupert, 1986; Pfanner *et al.*, 1987). For example, the import of the F_0F_1-ATPase subunit β ($F_1\beta$) was dependent on both $\Delta\psi$ and NTPs. Non-hydrolyzable ATP analogues were not able to fulfil the NTP requirement indicating that actual cleavage of a phosphodiester bond is necessary. It was demonstrated that the sensitivity of precursor proteins in reticulocyte lysate to non-specific proteases is greater in the presence of NTPs (Pfanner *et al.*, 1987). Thus, it was postulated that the hydrolysis of NTPs is involved in modulating the folding of precursors in the cytosol, and thereby conferring a conformation that is competent for import. A series of fusion proteins between the F_0-ATPase subunit 9 and mouse dihydrofolate reductase were constructed which demonstrated that the import of precursors having identical presequences, but different mature protein

parts, required different levels of NTPs. These results are consistent with the view that the NTPs are required to confer an import competent conformation on the precursor during the processes of recognition and binding to the mitochondrion (Pfanner *et al.*, 1987; Eilers *et al.*, 1987).

During or shortly following the translocation process, precursor proteins with *N*-terminal presequences are proteolytically processed by a metal-dependent processing peptidase located in the matrix (Böhni *et al.*, 1980; Miura *et al.*, 1982; Schmidt *et al.*, 1984; Hawlitschek *et al.*, 1988). The processing peptidase has recently been purified from *Neurospora crassa* (Hawlitschek *et al.*, 1988). The enzyme has been shown to consist of two polypeptides of 57 kDa and 52 kDa apparent molecular mass. The peptidase activity is associated with the 57 kDa component and it is thought that the 52 kDa component is required as an 'assistant protein' which functions to increase the catalytic rate and specificity of the processing reaction. It is also suggested that the 52 kDa component is equivalent to the product of the *mas-1* gene in yeast, a mutant of which lacks precursor processing activity at restrictive temperatures (Witte *et al.*, 1988).

Once specifically directed to mitochondria, imported precursor proteins must then be correctly sorted to their functional intramitochondrial location. Generally, sorting of precursors is accomplished by direct transfer to their correct mitochondrial subcompartments. In some cases, however, sorting occurs by a more sophisticated mechanism (for example, the precursor to the Fe/S protein of the ubiquinol–cytochrome *c* reductase complex, cytochrome b_2 and cytochrome c_1 (Hartl *et al.*, 1987), all of which function in the intermembrane space and contain a presequence which is processed in two distinct steps). In these cases, the precursors are first imported completely into the matrix where the first part of the presequence is removed by the processing peptidase to generate an intermediate-size product. This is then redirected back across the inner membrane to the intermembrane space where the second half of the presequence is proteolytically removed. Contrary to the stop-transfer model (van Loon & Schatz, 1987; van Loon *et al.*, 1987), hydrophobic stretches of amino acids in the second half of the prepiece appear to act as transport signals to direct export from the matrix into the intermembrane space (Hartl *et al.*, 1987).

In summary, the import of proteins into mitochondria involves a number of consecutive steps which act in concert to efficiently and specifically direct proteins to their correct intracellular and intramitochondrial location. While many of these steps are common for a large number of mitochondrial protein constituents (i.e. shared receptors, contact sites, $\Delta\psi$ and NTP requirements, proteolytic processing events), the overall import pathway often varies significantly between different proteins. Most of these variations are characteristic of the protein's final intramitochondrial location and the events which are necessary to deliver properly a protein to its correct functional location. The individual molecules involved in these processes are now the subject of current research interest.

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